

Product Information

Anti-NPTII (C-Terminal)

Produced in Rabbit, Affinity Isolated Antibody

Product Number **N 6412**

Product Description

Anti-NPTII (C-Terminal) is developed in rabbit using as immunogen a synthetic peptide corresponding to amino acids 250-264 located at the C-terminus of *E. coli* NPTII, conjugated to KLH. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-NPTII (C-terminal) recognizes bacterial NPTII expressed in transgenic tobacco plants and *E. coli*. Applications include the detection of NPTII by immunoblotting (30 kDa). Staining of NPTII in immunoblotting is specifically inhibited by the immunizing NPTII peptide (*E. coli*, amino acids 250-264).

The most commonly used selection markers in plant transformation are the bacterial neomycin phosphotransferase II (nptII) gene (neo) and the bar gene encoding the phosphinotricin acetyl transferase (pat).¹ The *nptII* gene, also known as the kanamycin-resistance marker gene, encoding for NPTII (30 kDa) an aminoglycoside 3'-phosphotransferase II (APHII), was isolated from the *E. coli* transposon Tn5.^{2,3} The *nptII* gene confers resistance to some aminoglycoside antibiotics including neomycin and kanamycin in bacteria and plant cells and to G418 sulfate in mammalian cells.^{4,5} NPTII phosphorylates and inactivates kanamycin, preventing kanamycin from binding to the 30S ribosomal subunit to inhibit protein synthesis, thus rendering cells resistant to the antibiotic.^{6,7} NPTII has been used routinely as a selection marker in the production of genetically engineered crops.⁸⁻¹³ It is an approved food additive for use as a processing aid in the development of new varieties of tomato, wheat, oilseed rape and cotton. Antibodies specific to NPTII can be used to detect relative transfection efficiencies in cells or whole plants where multiple transfections are performed in parallel with different plasmids.

Reagent

The antibody is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~1.0 mg/mL

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

By immunoblotting, a working antibody concentration of 1-2 µg/mL is recommended using an extract (cytosolic fraction from the leaves of transgenic *Nicotiana tabacum* expressing *E. coli* NPTII.

By immunoblotting, a working antibody concentration of 0.5-1 µg/mL is recommended using a whole cell extract of *E. coli* expressing NPTII.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

1. Miki, B., and McHugh, S., J. Biotechnol., **107**, 193-232 (2004).
2. Beck, E., et al., Gene, **19**, 327-336 (1982).
3. Mazodier, P., et al., Nucl. Acids Res., **13**, 195-205 (1985).
4. Le, T.T., et al., Neurogenetics, **3**, 7-16 (2000).
5. Osterhout, D.J., et al., J. Neurosci., **17**, 9122-9132 (1997).
6. Wright, G.D., et al., Adv. Exp. Med. Biol., **456**, 27-69 (1998).
7. Smith, C.A., and Baker, E.N., Curr. Drug Targets Infect. Disord., **2**, 143-160 (2002).
8. Fuchs, R.L., et al., Biotechnology, **11**, 1537-1542 (1993).
9. Curtis, I.S., et al., Meth. Mol. Biol., **49**, 149-159 (1995).
10. de Vries, J., and Wackernagel, W., Mol. Gen. Genet., **257**, 606-613 (1998).
11. Patnaik, D., and Khurana, P., E. J. Biotechnology (online), **4**, no. 3, (2001), available from: <http://www.ejbiotechnology.info>.
12. Schmidt, M.A., et al., Crop Sci., **44**, 963-967 (2004).
13. Goodwin, J.L., et al., Meth. Mol. Biol., **286**, 191-202 (2005).

KAA/ER 09/05

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.